DEVELOPMENT AND OPTIMISATION OF MULTI-NOZZLE SPRAY MANUFACTURING PROCESS FOR BLACK SEED OIL ALGINATE BEADS

BY

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ABSTRACT

Black seed oil (BSO) is a natural remedy derived from *Nigella sativa* Linn. The main active ingredient in BSO, thymoquinone, is well-known for its antibacterial and antioxidant properties. Prophet Muhammad (PBUH) stated that black seed is one of the remedies for all diseases except death. However, consumers find it difficult to take BSO on a daily basis due to its bitter taste. BSO's bitter taste is hidden by encapsulating it in a soft gelatine capsule. However, because of the capsule's size, children and the elderly have difficulty swallowing it. BSO is encapsulated in micrometre-sized alginate beads to increase palatability in individuals of all ages, particularly children and the elderly. This research aims to encapsulate BSO in alginate beads made with a multi-nozzle spraying method that has the same quality and characteristics as a single nozzle. From the results, the overhead stirrer combined with homogeniser and flow cell sonicator with a minimum amplitude of 20 produced the best droplet size, PdI, and dissolution time of BSO alginate nanoemulsion. The flow rate and applied voltage significantly influenced the diameter of BSO alginate beads (p-value < 0.05). The sphericity factor (SF) was unaffected by flow rate, applied voltage, or distance between the nozzle and the cross-linking solution. Thus, the optimisation of the mixing, emulsification and electrospraying process was successfully done on the lab scale. The first and fifth designs showed the best maldistribution factor (MF) value, near-zero MF value, in designing and analysing a 3D-printed pilot-size multi-nozzle prototype for the electrospraying process at the lab scale. The fifth design was chosen, and this is the first multi-nozzle with two baffles in the core, combining two different types of the best baffle designs. The results showed that a multi-nozzle spray produced a good SF value and beads size without utilising high voltage. Equipment involved in the BSO alginate beads manufacturing line was optimised at the IKOP® Sdn. Bhd pilot plant. The 100 L mixing tank equipped with stirrer and homogeniser at 500 and 1500 rpm speed, respectively was capable of reducing the droplet size. To process a greater volume with the least amount of energy, BSO alginate nanoemulsion was produced using flow configuration at the best process parameters obtained from pilot plant study. Using a 70% amplitude power rather than 90% to produce BSO alginate nanoemulsion at a flow rate of 72, 144, 216, or 288 mL/min is advisable due to less energy consumption. The critical quality attribute of BSO alginate nanoemulsion, which were particle size and PdI, was accomplished using both amplitude powers. The difference between the top and bottom of a 20-litre holding tank is 7.18 %, thus within in-process quality control (IPQC) target. From the data, 83.33 % of the beads have an SF of less than 0.05, which was above the IPQC's goal which is more than 75 % of the beads having an SF of less than 0.05. The IPQC beads diameter range is between 1.50 mm to 2.50 mm, and all BSO beads diameter readings are within that range. The multi-nozzle spray apparatus achieved successful outcomes by producing wet beads with an SF of less than 0.05. Hence, the multi-nozzle spray machine can manufacture BSO alginate wet beads in large quantities. The curing with filter tank placed at IKOP® Sdn. Bhd. was capable of curing and filtering the BSO alginate beads production process where it met the goal of a continual decrease in calcium ion concentration. Finally, this study successfully developed and optimised the multi-nozzle spray production line in producing BSO alginate beads with same quality and characteristics as a single nozzle in the lab scale.

خلاصة البحث

زيت الحبة السوداء هو علاج طبيعي مستمد من النيجيلا ساتيفا لين.المكون النشط الرئيسي في زيت الحبة السوداء، الثيموكينون،معروف جيدا بخصائصه المضادة للأجسام ومضادات الأكسدة.ذكر الرسول صلى الله عليه وسلم أن الحبة السوداء هي واحدة من وسائل العلاج لجميع الأمراض باستثناء الموت.ومع ذلك،يجد المستهلكون صعوبة في تناول زيت الحبة السوداء يوميا بسبب طعمه المر.ويغلف زيت حبة السوداء في خنافس ألجينات بحجم مايكرومتر لزيادة قابليتها للتحجيم لدى الأفراد من جميع الأعمار،ولا سيما الأطفال والمسنين.ويهدف هذا البحث إلى تغليف زيت الحبة السوداء في خنافس الألجينات المصنوعة بطريقة الرش المتعدد الفوهات التي لها نفس نوعية وخصائص فوهة واحدة.واستنادا إلى النتائج،فإن المثير العام جنبا إلى جنب مع متجانس وأجهزة صوتية خلية التدفق مع الحد الأدنى من سعة 20 أنتجت أفضل حجم قطرة، PDI،ووقت انحلال زيت الحبة السوداء الجينات نانوميدات.وقد أثر معدل التدفق والفولتية التطبيقية تأثيراً كبيراً على قطر خنافس ألجينات زيت الحبة السوداء ($p\text{-value} < 0.05$).ولم يتأثر معامل التحسس بمعدل التدفق،أو الجهد المطبق،أو المسافة بين الفوهة ومحلول الربط العابر.وأظهر التصميمان الأول والخامس أفضل قيمة لمعامل سوء التوزيع،وهي قيمة لمعامل سوء التوزيع تقريبا من الصفر،في تصميم وتحليل ثلاثة أبعاد مطبوعة نموذجا تجريبيا متعدد الفوهات لعملية الرش الكهربائي على مقياس المختبر.التصميم الخامس تم اختياره،وهذا هو أول متعدد الفوهات مع اثنين من الحافتين في النواة، والجمع بين نوعين مختلفين من أفضل التصاميم.وأظهرت النتائج أن رذاذ متعدد الفوهات ينتج قيمة جيدة لعامل الثبات وحجم الخرز دون استخدام الجهد العالي.وكانت جميع المعدات المستخدمة في خط تصنيع خرز زيت الحبة السوداء مؤهلة في مصنع شركة IKOP® Sdn. Bhd.وكشفت النتائج أن خزان خلط 100 لتر المجهز بسرعة 500 و 1500 rpm،على التوالي،كان قادرا على تقليل حجم القطرة. و من أجل معالجة حجم أكبر بأقل كمية من الطاقة،تم إنتاج مستحلب نانوميدات زيت الحبة السوداء باستخدام تشكيل التدفق في أفضل بارامترات العملية التي تم الحصول عليها من الدراسة التجريبية للنبات.استخدام طاقة سعة 70%بدلا من 90% لإنتاج زيت الحبة السوداء الجينات نانوميدات بمعدل تدفق من 72, 144, 216،أو 288 مل/مين مستصوب بسبب استهلاك أقل للطاقة.تم إنجاز الصفة الحرجة للنوعية لزيت الحبة السوداء الجينات نانوميدات،التي كانت حجم الجسيمات و PDI،باستخدام كل من قوة التعمق.والفرق بين أعلى وأسفل خزان الإمساك بسعة 20 لتراً 7.18%،وبالتالي ضمن هدف مراقبة الجودة أثناء العملية.ووفقاً للنتائج،فإن 83.33% من الخرز لديها عامل الثبات أقل من 0.05،وهو ما كان أعلى من هدف مراقبة الجودة أثناء العملية،وهو أكثر من 75% من الخرز التي لديها عامل الثبات أقل من 0.05.يتراوح قطر خرز التحكم في مراقبة الجودة أثناء العملية بين 1.50 مم و 2.50 مم،وجميع قراءات قطر خرز الحبة السوداء تقع في هذا النطاق.وحقق جهاز الرش متعدد الفوهات نتائج ناجحة بإنتاج خرز رطب يقل فيه عامل الثبات عن 0.05.وأظهرت النتائج أن المعالجة بخزان الترشيح الذي وضع في شركة IKOP® Sdn. Bhd. كانت قادرة على معالجة وتصفية عملية إنتاج خرز زيت الحبة السوداء.وقد حقق الهدف المتمثل في الانخفاض المستمر في تركيز أيونات الكالسيوم إلى أن يتم تحقيق هضبة من أجل عملية العلاج.وفي الختام، نجحت هذه الدراسة في تطوير خط إنتاج الرذاذ المتعدد الفوهات في إنتاج خرز زيت الحبة السوداء بنفس نوعية وخصائص فوهة واحدة في مقياس المختبر.

APPROVAL PAGE

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DECLARATION

I hereby declare that this thesis is the result of my own investigations, except where otherwise stated. I also declare that it has not been previously or concurrently submitted as a whole for any other degrees at IIUM or other institutions.

Shaiqah Binti Mohd Rus

Signature ..... Date 28/02/2022

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LIST OF ABBREVIATION

BSO	Black Seed Oil
CE	Coarse Emulsion
CFD	Computational Fluid Dynamic
CPPs	Critical Process Parameters
CQAs	Critical Quality Attributes
DOX	Doxorubicin
DQ	Design Qualification
EHDA	Electrohydrodynamic Atomisation
EQ	Equipment Qualification
FAT	Factory Acceptance Test
FC	Flow Configuration
GMP	Good Manufacturing Practice
H	Homogeniser
IPQC	In Process Quality Control
IQ	Installation Qualification
MF	Maldistribution Factor
MS	Magnetic Stirrer
NS	*Nigella sativa*
OQ	Operational Qualification
OS	Overhead Stirrer
OSH	Overhead Stirrer Combined with Homogeniser
PdI	Polydispersity Index
PQ	Process Qualification
RSM	Response Surface Methodology
SA	Sodium Alginate
SF	Sphericity Factor
STL	Standard Tessellation Language
TQ	Thymoquinone
T80	Tween 80

CHAPTER ONE

INTRODUCTION

1.1 BACKGROUND OF THE STUDY

Present sedentary and long working hours are one of the possible human explanations for their inability to maintain a balanced diet. This lifestyle encourages researchers to boost nutrient levels in natural products to enhance human health (Singh et al., 2018). *Nigella sativa* (NS), also known as black seed or black cumin, has been extensively studied because of its antioxidant, antibacterial, anti-inflammatory, and anti-cancer properties owing to its active ingredient, which is thymoquinone (TQ) (Majdalawieh & Fayyad, 2016; Woo et al., 2011). Black seeds consist of nutritional components such as vitamins and proteins, together and after modification such as TQ, thymol, thymohydroquinone, nigellicine, nigellimine, and α-hederin, which encourage the increasing number of phytochemicals and pharmacological studies (Anandan et al., 2017). However, stability investigation on TQ showed a low stability profile in all aqueous solutions with fast degradation that varied with the solvent category, mainly at an alkaline pH (Salmani et al., 2014). It had also been pointed out that aqueous solutions is not suitable as therapeutic vehicles for TQ preparations (Salmani et al., 2014). The encapsulation of BSO in alginate beads is intended to solve the problem of bitterness, transportation issues, stability, and the difficulty of swallowing soft gel capsules containing BSO by children and the elderly. Furthermore, market survey conducted in the United States found that out-of-pocket expenditures on natural product supplements were approximately $12.8 billion in 2012 (Nahin et al., 2016). Meanwhile, retail sales of vitamins and nutritional supplements in Malaysia have more than doubled in the last

decade, from RM940.4 million (USD248.3 million) in 2005 to RM1.9 billion (USD572.6 million) in 2014 ("Dietary Supplements in Malaysia," 2021). To ensure that the BSO alginate bead product meets the market demand, the process needs to be scaled up using a multi-nozzle spray. However, pilot-scale multi-nozzle spray for alginate is not commercially available as a production line for pharmaceutical products. To establish a new production line that meets pharmaceutical Good Manufacturing Practice (GMP), it requires enormous activities of qualifications of the equipment and verification of the process. The BSO-alginate bead production in a larger scale is expected to meet the increasing demand from the consumers.

1.2 PROBLEM STATEMENT

BSO is readily available in liquid form, which is associated with issues such as bitterness, transportation, and stability. These issues spark researchers' and manufacturers' attention to encapsulate BSO into different dosage forms, such as soft gel capsule. Even though the capsule can solve the issues as mentioned earlier, children and the elderly are facing with difficulty in swallowing the BSO capsules. As far as this study concern, there is no product commercially available in the market that encapsulates BSO into alginate beads in micrometre size which is produced by GMP plant.

The production of BSO in alginate beads in the large quantity requires the use of multi-nozzle for the spraying process because the single nozzle cannot uphold the large-scale and continuous process. As for now, the multi-nozzle parts for spraying the BSO in alginate bead production are still not commercially available. As far as this study is concerned, the multi-nozzle spray is only designed for the production of the

nanoparticle. Multi-nozzle spray for the production of millimetre size of beads is still not available in the market.

Thus, it is essential to design a multi-nozzle with specific considerations, such as laminar flow and turbulent flow of the emulsion inside the multi-nozzle, which gives an equal distribution for each nozzle that will involve 144 nozzles in the production. There are several problems associated with a multi-nozzle electrospray, such as increasing the flow rate, which will lead to an electrical shortage. This problem shows that the microfluidic system has to be redesigned in order to reduce the interaction between different nozzles.

Additionally, equipment qualification (EQ), especially for multi-nozzle spraying process, is still not established until now for real industrial processes. Besides, the process qualification (PQ) of BSO alginate beads will also need to be done following the GMP criteria. The production of BSO in alginate beads using a multi-nozzle that will be established at the lab-scale will be compared against the pilot-scale to ensure that the targeted specification is achieved.

1.3 RESEARCH HYPOTHESIS

The encapsulated BSO in alginate beads production, which will be produced by multi-nozzle spraying process are of the same quality and characteristics as a single nozzle in the lab scale.